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TRACE ELEMENTS AND NANOMATERIALS
RISK AND HEALTH IMPACT ASSESSMENT

SILVER-BASED BIOCIDES AND TITANIUM DIOXIDE PARTICLES IN FACE MASKS FOR GENERAL USE

Final report of the TiO_2Mask and AgMask COVID-19 projects
February 2023

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For this, Sciensano builds on the more than 100 years of scientific expertise of the former Veterinary and Agrochemical Research Centre (CODA-CERVA) and the ex-Scientific Institute of Public Health (WIV-ISP).

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Chemical and physical health risks - Trace elements and nanomaterials

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EXECUTIVE SUMMARY

Wearing face masks is an important and widely applied public health measure to control infectious agents. Textile companies propose new solutions to the challenges associated with, for example, the COVID-19 pandemic, incorporating specific nanofibre, nanocomposite and nanoparticle technology into face masks, including the application of silver-based biocides. Also, titanium dioxide (TiO_2) nanoparticles are widely applied in synthetic fibres during the production process, as a white colorant, as a matting agent, to assure durability reducing polymer breakdown by ultraviolet light, and also as functionalizing agent to give various enhancing properties to the textiles. Face masks have undoubtedly proven useful as a protective measure against various infectious agents, and in particular in combating the COVID-19 epidemic. Hence, we do not call for people to stop wearing face masks. However, the properties of silver-based biocides and of TiO_2 particles are not imperative for a face mask to work properly. As certain concerns about the safety of these substances remain, the benefits of their use must be balanced against possible toxicity due to inhalation exposure.

In this perspective, in depth research of (nano)technology applications in textiles, and specifically the added value of biocidal silver treatment of face masks (examined in the AgMask project) and the application of TiO_2 particles (examined in the TiO_2Mask project) is important to avoid possible future consequences caused by poorly regulated use of these substances.

A first and important step is the development of detection and characterisation methods. To evaluate the presence and amounts of silver-based biocides and TiO_2 particles in face masks, an approach was set up that combines total silver (Ag) and titanium (Ti) measurement using ICP-MS or ICP-OES with *in situ* analysis of silver-based biocides and TiO_2 particles in ultra-thin sections of face masks using STEM and EDX. This allowed to demonstrate the presence of TiO_2 particles in the synthetic fibres and varying amounts and different types of silver-based biocides in a selection of face masks available on the Belgian market and intended to be worn by the general public. The following types of silver-based biocides were demonstrated: (i) Ag^+ ions, (ii) metallic Ag^0 nanoparticles (NP) distributed in the matrix of the fibres, (iii) Ag NP and large silver particles at the surface of, or close to cotton fibres in face masks containing polycationic polymers binding Ag^+ ions (Silvadur™ technology), (iv) coatings consisting of metallic silver releasing Ag^+ ions, Ag^0 NP and large silver particles. The detailed protocols, which are considered useful tools to implement for (regulatory) quality control of face masks, have been published publicly.

Complementary to the *in situ* TEM imaging of TiO_2 particles in cross-sections of synthetic fibres, a new method was developed which allows polyamide and polyester fibres of face masks and other textiles to be dissolved, thus enabling to measure the number-based size distribution of (constituent) TiO_2 particles from polyester and polyamide fibres in face masks more efficiently and precisely. This method can be used in risk analysis and contribute to implementing existing regulations.

The assessment of potential risks associated with the inhalation exposure of silver-based biocides and TiO_2 particles released from face masks during normal use, is constrained by the lack of available data on release and exposure. Therefore, a conservative approach comparing the measured Ag and TiO_2 content of the face mask with limit values, was applied to evaluate the safety of the face masks with a minimum of assumptions. It allows to differentiate masks that can be considered intrinsically safe (safe-by-design) from those that require a more extensive assessment. The latter requires information on the modalities of use (duration, frequency of change, washing, …) by the intended population, and on the release of silver-based biocides and/or TiO_2 particles from the face mask during use, which may lead to inhalation exposure.

The safe amount of TiO_2 at the surface of the textile fibres is estimated to be 3.6 µg per mask. For all examined face masks, the amount of TiO_2 particles at the surface of the textile fibres notably exceeds this safety limit, indicating that further risk analysis is required.

For silver, two limit values are considered, based on occupational exposure limits (OEL) that are defined by the two forms of silver-based biocide. More specifically a generic limit of 25 µg for silver occurring as metal dust, fume, and soluble compounds, and a specific limit of 2.25 µg for silver occurring as nanoparticles. More than half of the analysed face masks that contain detectable amounts of silver, contain levels well below the relevant limit values and can be considered intrinsically safe. Several face masks contain, however, levels of silver that exceed one or both of the limit values used in this study, and a final conclusion (intrinsically safe) regarding the safety of these masks could not be established.

In this perspective, the scarcity of quantitative (measured or modelled) information about the release of TiO_2 particles and silver-based biocides from face masks while using them is a major limitation for the safety assessment of these substances. There is hardly any of such information available in literature. Aiming for a quantitative estimation of this release, new methods were explored to measure particle release in conditions that mimic real-life use. In addition, existing methods for quality control of textile, including conventional abrasion test methods and leaching experiments, were also evaluated.

An experimental set-up was developed in collaboration with VITO to evaluate the release of Ag and TiO_2 particles from selected face masks mimicking real-life breathing conditions. Overall, the measurements, which are in the order of the detection limits, suggest that the amounts of TiO_2 and Ag released from the examined face masks are low. Drawing definitive conclusions about particle release and associated risks based on this experimental set-up remains difficult. It requires a more extended validation, ensuring that released particles are effectively captured, detected and distinguished from contamination.

Conventional rubbing and abrasion tests, typically used to test the quality of textile materials, were screened but were found to be neither suitable to simulate the wear and tear of face masks nor to detect the subsequent release of TiO_2 nanoparticles. The techniques were not tested for silver-based biocides.

Leaching experiments were evaluated as a relatively simple, alternative method to estimate the release of Ag and Ti (as a proxy for TiO_2) from face masks. The applied leaching conditions, combining the application of chemical stress (i.e. acidic pH of the artificial sweat solution) and physical stress (i.e. end-over-end shaking), can be considered more stringent than those occurring during normal use. Hence, leaching experiments are proposed as an alternative and relatively cheap method to evaluate the potential release of selected chemicals, and as a higher tier method to refine the risk assessment if face masks are not safe-by-design.

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ABSTRACT

Wearing face masks is an important and widely applied public health measure to control infectious agents. The benefits of the use of silver-based biocides or TiO_2 particles in face masks have to be balanced against their possible inhalation toxicity to avoid possible future consequences caused by a poorly regulated use.

The presence and amounts of silver-based biocides and of TiO_2 particles in face masks were demonstrated using a new approach that combines total Ag and Ti measurement using ICP-MS or ICP-OES with *in situ* analysis of silver-based biocides and TiO_2 particles in ultra-thin sections of face masks using STEM and EDX. It allowed to demonstrate the presence of TiO_2 particles in the synthetic fibres and varying amounts and different types of silver-based biocides in a selection of face masks offered on the Belgian market and intended to be worn by the general public. A new and complementary method allowed dissolving polyamide and polyester fibres of face masks and other textiles, allowing to measure the number-based size distribution of (constituent) TiO_2 particles more efficiently and precisely in view of existing regulations and for risk analysis.

A major limitation for the safety assessment of TiO_2 particles and silver-based biocides in face masks is the scarcity of quantitative (measured or modelled) information about their release during the use of face masks. An approach comparing the measured Ag and TiO_2 content of the face mask with limit values, was applied to evaluate the safety of the face masks with a minimum of assumptions regarding such release. It allows to differentiate masks that can be considered intrinsically safe (safe-by-design) from those that require a more extensive assessment. An experimental set-up developed in collaboration with VITO to evaluate the release of Ag and TiO_2 particles from selected face masks mimicking real-life breathing conditions, suggests that the amounts of TiO_2 and Ag released from the examined face masks are low. The methodology needs, however, to be further validated before definite conclusions can be drawn. Conventional rubbing and abrasion tests, typically used to test the quality of textile materials, were found unsuitable to evaluate the release of TiO_2 nanoparticles. Leaching experiments can be useful as a relatively cheap method to evaluate the potential release of selected chemicals, and as a higher tier method to refine the risk assessment when face masks are not safe-by-design.

ABBREVIATIONS

Ag	Silver
CLP	Classification, Labelling and Packaging
DNEL	Derived-No-Effect-Level
ECHA	European Chemicals Agency
EDX	Energy Dispersive X-ray Spectroscopy
EM	Electron Microscopy
Fmin	Minimum Feret Diameter
HEPA	High Efficiency Particulate Air
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IME	Fraunhofer Institute for Molecular Biology and Applied Ecology
ISO	International Organization for Standardization
JRC	Joint Research Centre
LOQ	Limit of Quantification
MCE	Mixed Cellulose Ester
NP	Nanoparticle
OEL	Occupational exposure level
pH	Potential of Hydrogen
SEM	Scanning Electron Microscopy
STEM	Scanning Transmission Electron Microscopy
TEM	Transmission Electron Microscopy
Ti	Titanium
TiO_2	Titanium dioxide
UV	Ultraviolet

RESULTS REPORTED IN THE INTERMEDIATE AgMask AND TiO_2Mask REPORTS

Introduction

Wearing face masks was adopted globally as a public health measure to reduce the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and it remains an important medical device to control infectious diseases in general. During the COVID-19 crises, the demand for face masks increased rapidly, and due to shortages of surgical-grade face masks, many single-use and reusable face masks made from a variety of fabrics and materials became available on the market. To overcome the challenges associated with the pandemic, textile companies produced face masks from materials that incorporated nanotechnology in order to offer masks of enhanced breathability and filtration capacity or with antiviral and antibacterial properties[1,2].

Many types of reusable face masks that were available on the Belgian market, claimed antimicrobial properties[3–7] relying on the incorporation of silver-based biocides in the materials. The antimicrobial effect of silver[8–10] has been extensively studied, hence its application in face masks seemed an attractive selling point for consumers. However, literature suggests that exposure to silver, particularly in the form of nanoparticles, can negatively impact health, depending on the type of silver-based biocide, its stability during application and cleaning, and the type of exposure of the users[11–13]. Although functionalized textiles may offer benefits because of their antimicrobial properties, concerns about their safety remain and, hence, these benefits must be balanced against possible toxicity due to inhalation exposure.

In this context, Sciensano initiated two COVID-19 research projects: *(i)* the AgMask project which aimed to evaluate the types of silver-based biocides and silver concentrations in face masks available on the Belgian market and their potential health risks due to inhalation exposure and *(ii)* the TiO_2Mask project that focused on the identification, physicochemical characterisation and the estimation of release of titanium dioxide (TiO_2) particles from face masks. The second project was started because a notable amount of TiO_2 particles was observed during the first series of electron microscopy analyses (in the context of the AgMask project). This observation was unexpected as there was no indication from the producers on the packaging about the presence of TiO_2 nanoparticles in the face masks. Considering that TiO_2 is classified as a carcinogen when inhaled, Carc. 2, H351 (inhalation)[14] [15], knowledge on its presence and release in face masks was considered necessary for the general public and for regulatory bodies.

Summary of the intermediate results of the AgMask and TiO_2Mask studies

The intermediate report of the AgMask project described the evaluation of the types, efficient use and health risks of application of silver-based biocides to provide antimicrobial properties to face masks[16]. The results are described and discussed in more detail in the peer-reviewed publication "Application of silver-based biocides in face masks intended for general use requires regulatory control" by Mast et al. (2023)[17]. Summarizing, this study optimized methodologies to characterise silver-based biocides directly in the face masks, by measuring their total silver content using ICP-MS and ICP-OES based methods, and by visualizing the type(s) and localization of silver-based biocides using electron microscopy based methods. Thirteen out of 20 selected masks intended for general use, contained detectable amounts of silver ranging from 3 µg to 235 mg per mask. Four of these masks contained silver nanoparticles, of which one mask was silver coated. Comparison of the silver content with limit values derived from existing inhalation exposure limits for both silver ions and silver nanoparticles, allowed to differentiate safe face masks from face masks that required a more extensive safety assessment. These findings urged for in-depth characterisation of the applications of silver-based biocides and for the implementation of regulatory standards, quality control and product development based on the safe-by-design principle for nanotechnology applications in face masks in general.

The intermediate report of the TiO_2Mask project described the identification, physicochemical characterisation and preliminary risk analysis of TiO_2 particles in face masks.[18] The results are described and discussed in more detail in the peer-reviewed publication of Verleysen et al.[19]. Summarizing, fibre-grade TiO_2 (nano)particles were demonstrated in synthetic textile fibres of face masks intended for the general public. STEM-EDX analysis on sections of a variety of single-use and reusable face masks visualized agglomerated near-spherical TiO_2 particles in non-woven fabrics, polyester, polyamide and bi-component fibres. Median sizes of constituent particles ranged from 89 to 184 nm, implying an important fraction of nano-sized particles (< 100 nm). The total TiO_2 mass determined by ICP-OES ranged from 791 to 152345 µg per mask. The estimated TiO_2 mass at the fibre surface ranged from 17 to 4394 µg per mask, and systematically exceeded the acceptable exposure level of TiO_2 by inhalation (3.6 µg), determined for a scenario where face masks are worn intensively. The importance of wearing face masks against COVID-19 is unquestionable. Even so, these results urge for in-depth research of (nano)technology applications in textiles to avoid possible future consequences caused by a poorly regulated use and to implement regulatory standards phasing out or limiting the amount of TiO_2 particles, following the safe-by-design principle.

So far, the results reported for both projects relied on the powerful combination of electron microscopy techniques for *in situ* localization of silver-based particles and for the determination of its chemical form in combination with spectrometry analysis (i.e. ICP-MS) for elemental quantification. The developed approach, summarized in Figure 1, allowed to identify face masks containing silver-based biocides and/or TiO_2 particles and to differentiate masks that can be considered intrinsically safe from those that require a more extensive assessment. Detailed protocols related to the developed methodology have been published in detail by Wouters et al.[20] and Mercier et al.[21] on Research Square, the open access platform for methods, supported by the Springer-Nature publishing group. They are considered as useful tools for implementation of (regulatory) quality control of face masks.

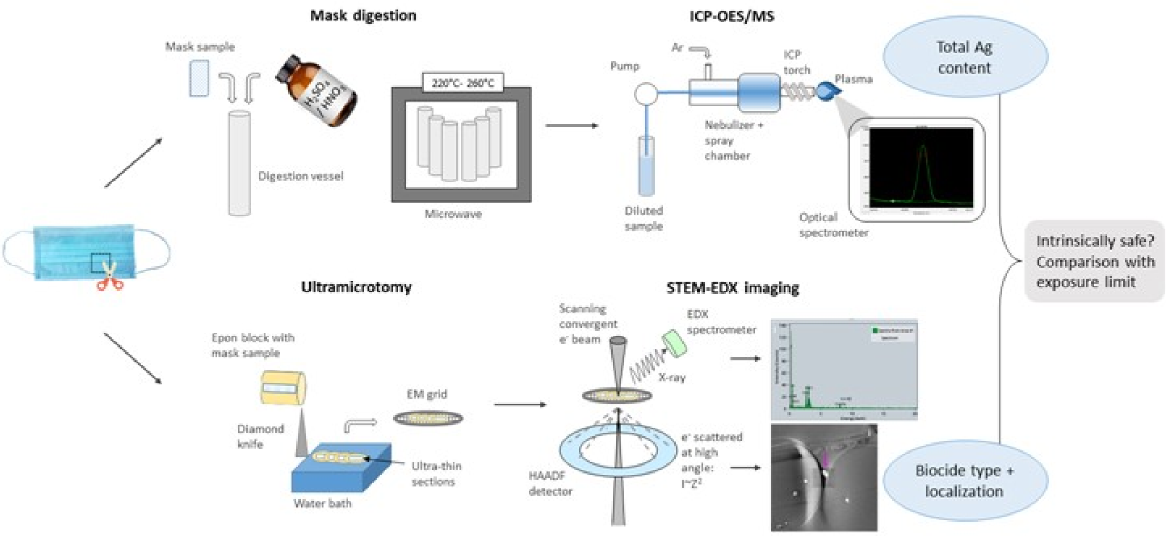

Figure 1 Overview of the methodology applied to evaluate silver-based biocides and TiO_2 particles in face masks.

Rationale of continued research

In the studies described by Mast et al.[17] and Verleysen et al.[19], minimal assumptions were made about the likelihood of the release of silver-based biocides and TiO_2 particles because direct measurement of such release to estimate exposure is problematic. Although the reported physicochemical characterisation of silver-based biocides and TiO_2 particles applied in face masks provides essential information for risk assessment, a conventional risk assessment, combining exposure with hazard assessment, could not be realized because no methods are available for direct measurement of their release and inhalation uptake when face masks are worn. Hence, an indirect approach was followed for the risk assessment, in which the total amount of Ag and TiO_2 measured in the face masks was compared with their respective threshold values for inhalation toxicity. This approach revealed that several face masks required a more extensive safety assessment, preferably taking release into account. Supporting on the results obtained in the preliminary reports, different approaches to estimate the release of silver-based biocides and TiO_2 particles from face masks, were further investigated.

Since inhalation is considered to be the main route of exposure to silver-based biocides and TiO_2 particles from wearing face masks, an experimental set-up that simulates breathing was tested, in collaboration with VITO, to evaluate any release of Ag and TiO_2 particles from face masks under conditions that represent real wearing of face masks. The information from the breathing experiments should provide direct exposure data for risk assessment.

Additionally, alternative methods were explored to evaluate the release of silver-based biocides and TiO_2 particles from face masks. In collaboration with the Department of Materials, Textiles and Chemical Process Engineering of the University of Gent, conventional abrasion methods, commonly used in the textile industry for quality control, were investigated to estimate release of TiO_2 particles.

A “wet” technique that estimates release based on leaching in an artificial physiological solution, was also evaluated as a simple method to determine migration of silver-based biocides and TiO_2 particles from face masks. Based on the relatively harsh conditions to which the face masks are exposed during leaching, this method can be considered as a worst case scenario for release by inhalation.

The data obtained from the alternative methods to evaluate release (abrasion, leaching) can be used to contribute to the refinement of the preliminary risk assessment, described in the intermediate reports for AgMask and TiO_2Mask projects.

ADAPTING CONVENTIONAL ABRASION METHODS TO ESTIMATE RELEASE OF TiO_2 PARTICLES

Introduction

Because of its catalysing effect on the polymerization process, TiO_2 is typically used as an additive during synthetic polymer production of synthetic fibres[22]. As a result, commercial face masks (surgical or cloth), which are most commonly made of synthetic fibres, are likely to contain some level of TiO_2 nanoparticles. In addition, TiO_2 can be added in larger amounts to act as a functionalizing agent for protective or other special properties of the end-product (i.e. matting agent, UV protection of textile fibres, antimicrobial, antistatic agent)[23]. Crucial parameters related to the amount and potential danger of the released particles are defined as the location of the particles within or on the surface of fibres, the type and stability of the bond between particle and fibre, and the medium in which release takes place. During the different stages of the lifecycle of a mask, exposure to a variety of stresses, fluids/solvents or other impulses can possibly cause release of TiO_2 particles[24,25]. So far, literature is limited to work specifically studying the release of TiO_2 particles from textiles during washing conditions. Such studies show that only small percentages of the total TiO_2 content are typically released[26–29]. Evaluation of release of TiO_2 particles from face masks under different use conditions is largely lacking in literature.

Therefore, in collaboration with the Department of Materials, Textiles and Chemical Process Engineering at the University of Gent, the objective was to develop a test method to simulate and measure the release of TiO_2 particles from face masks during normal use. After a literature study, different conventional abrasion methods to simulate the wear and tear of face masks and to quantify the amount of released particles were evaluated.

Screening of abrasion methods

Existing rubbing and abrasion tests typically used for textile materials were screened in order to evaluate their suitability for simulation of wear and tear of face masks and their potential to induce release of TiO_2 nanoparticles. Four types of equipment (pilling box, washing testers, Crockmeter and Martindale tester) were tested and the effects on face masks were visually inspected.

Washing was not selected for final testing because it is not relevant for single-use masks and it is specific for release in the liquid phase. For the pilling box test ('ISO 12945') the risk of contamination was estimated to be the highest of all tested methods and therefore was not selected for further testing. The Crockmeter and Martindale tests were selected as the most practical and efficient for our purpose. In both cases an abradant is rubbed against the fabric with pressure and a rubbing motion is applied. The Crockmeter ('ISO 105-X12'), which simulates unidirectional friction, showed clear effects in terms of hairiness and fibrillation of filaments on single-use masks but was considered 'too soft' for reusable masks. The harsher Martindale test ('ISO 12947' and 'ISO12945') simulates a more random motion and is able to produce visible wear and tear effects on reusable masks but it was deemed too harsh for single-use masks.
Based on these evaluations, the following settings were selected for the release experiments:

- Crockmeter for single-use masks: 100x rubbed using a square foot with extra weight
- Martindale test for reusable masks: 5000 turns (9 kPa)

Estimation of release of TiO_2 particles by conventional abrasion methods

RESULTS OF ABRASION TESTS

A selection of masks, AgMask-05, AgMask-03 and AgMask-15, with low, intermediate and high TiO_2 contents, respectively, were used for testing release upon abrasion and methods for detection of release.

Scanning electron microscopy (SEM) was used to visualize the presence of (nano)particles on the fibre surface. This technique requires no specific sample preparation, Other than deposition of a nano-layer of gold coating, and it allows a longitudinal view of the fibres within the textile. In all three masks, particles of bright intensity could be observed inside the synthetic fibres. the particles are distributed all over the clearly embedded within the fibre diameter and they ar not present in the cotton fibres, (some layers of AgMask03 consist of a mix of polyester and cotton).
EDX was not available to check the chemical composition of particles. Still, based on their position in the fibre, on their size and shape properties detected particles were presumably identified as TiO_2.
Since EDX was not available to check the chemical composition of particles, detected particles were presumably identified as TiO_2 based on their position, size and shape. Only supporting on a visual inspection of the images and lack of compositional data, did not allow to make a correlation between the number of particles measured by SEM and the TiO_2 content as quantified before by ICP-OES[20,31].

From different textile abrasion techniques, the Crockmeter and Martindale abrasion methods were selected to test two types of face masks: disposable AgMask05 with relatively low TiO_2 content and washable mask AgMask15 with relatively high TiO_2 content. A comparison of SEM images before and after abrasion was done to assess possible release of TiO_2 from the fibre surface. For both masks, some alterations to the fibre structures, such as change in position and orientation of filaments and detachment of thermo-bonds could be observed after abrasion, but there was no visible difference in the particle content beneath the fibre surface. Even though these first tests do not indicate a strong particle release, a more in-depth investigation, including EDX and particle counting, is necessary to make reliable conclusions.

Preliminary investigations on the applicability of infrared spectroscopy for quantifying TiO_2 release from masks, remained unsuccessful. To that end, AgMask-05 was incinerated at high temperatures (800°C) and the major chemical components of the remaining ashes were analysed by infrared spectroscopy. A peak related to TiO_2 could be distinguished but the signal was too low for quantification.

LACK OF REFERENCE FIBRE MATERIALS

The importance of reference fibre materials, containing known concentrations and size distributions of TiO_2, as well as negative controls, i.e. synthetic fibres without TiO_2 embedded, was also addressed. At present, such reference materials do not exist or are not available on the market. In the pursuit of suitable reference yarns, a comparative study was carried out between a glossy and dull polyester yarn attempting to relate the glossiness to the amount of TiO_2 particles present on the fibres as measured by SEM. However, observed particle concentrations were too low in all samples to distinguish them from contamination. In addition, other differences in the fibre structure (e.g. cross section shape, texture) of the glossy and dull yarn were observed, hence no conclusions could be made. Therefore, in view of future method development and validation, the availability of reference fibre materials is a must.

Conclusions

A literature study showed that TiO_2 nanoparticles are widely applied in synthetic fibres during the production process but also as functionalizing agent to give various enhancing properties to the textiles. However, studies investigating the possible release of TiO_2 particles are largely lacking. Conventional abrasion methods, typically applied to test the quality of textiles, were evaluated to simulate release of TiO_2 from face masks as a result of friction (mimicking normal wear and tear). Although the abrasion methods clearly had an effect on the fibre structure, the applied detection methods (SEM, infrared spectroscopy) were at this stage not suitable to make any definite conclusion on release of particles. The application of these methods to measure release should be further developed. Related to that, in regard of method validation, the need for development of reference fibre materials was underlined.

EVALUATION OF RELEASE MIMICKING NORMAL BREATHING

Introduction

Literature data suggests that exposure to silver, particularly in the form of nanoparticles and by means of inhalation, can be unhealthy.[11–13,30] The results of *in vivo* studies demonstrate the induction of a pro-inflammatory response in the lungs upon inhalation of silver nanoparticles and also translocation into the bloodstream with effects in different organs.[12] In this context, the question of whether the use of silver (nanoparticles) in mouth masks leads to inhalation exposure is relevant. Depending on this exposure, a pro-inflammatory response can also be expected in humans, especially with intensive use. On top, a (negative) interaction with an infectious inflammatory reaction of, for example, Covid-19 is possible.[30] Currently, no data exist demonstrating silver (nanoparticles) release while wearing silver containing mouth masks, but the absence of release (e.g. tested as a quality parameter) has not been demonstrated either. Likewise, no information regarding the amounts of TiO_2 particles that are released and inhaled from face masks in normal and intensive use conditions is available yet. This information is however important because TiO_2 has been classified as a carcinogen when inhaled, Carc.cat.2, H351[14] according to the CLP classification.[15]

The possible release of silver and TiO_2 in the form of nanoparticles from mouth masks resulting in exposure by inhalation is an important knowledge gap. As an attempt to fill that gap and work towards a formal risk assessment, an experimental set-up for simulating breathing was developed in collaboration with VITO to evaluate the release of silver and TiO_2 particles from selected face masks under mimicked real life conditions.

Materials and Methods

BREATHING SET-UP AND MASK SELECTION

Face masks were mounted onto a Sheffield dummy head, as shown in **Error! Reference source not found.**. Breathing through the mask was simulated using a breathing machine capable of producing a continuous sinusoidal cycle of 25 cycles/min and 2.0 l strokes. This breathing machine is used in the simulating wearing test described in the standard EN 149:2001+A1:2009[31]. The artificial trachea tube of the dummy head is designed to have a separate inhalation and exhalation flow. Inhaled air samples were collected between the head and the breathing machine using filters and positively or negatively charged EM-grids. The experiment was executed in a HEPA-filtered, particle-free test chamber to prevent contamination from other sources.
AgMask-03, AgMask-08 and AgMask-15 were used to assess the release of particles upon simulated breathing. These experiments were conducted in triplicate. The total amount of silver and TiO_2 and their presence in nanoparticulate form was determined beforehand by ICP-OES/ICP-MS and TEM, respectively (Table 2). AgMask-15 and AgMask-08 (silver coating) represent worst cases due to the highest measured TiO_2 and Ag contents, respectively. A cotton mask, barely/not containing TiO_2 or silver, was used as negative control. To create positive control masks, negative control masks were spiked with nanoparticle dispersions containing TiO_2 (NM100, JRC) or Ag (NM300K, IME) representative test materials.

Figure 2: Experimental set-up with silver coated AgMask-08 onto dummy head connected to the breathing machine via the inhalation tube, filter holder and two MPS for sampling on a positively and negatively charged grid.

MEASURING PARTICLE RELEASE USING ICP-MS AND TEM

An initial set of experiments was performed to check the ability of detection from masks containing TiO_2 and Ag compared to negative control masks for various experimental set-up conditions. Different filters (Tissuquartz vs. MCE) were tested and particles were sampled from the air flow on the EM grids using either a Nanometer Aerosol Sampler (TSI, Shoreview, Minnesota, USA), or a Mini Particle Sampler (Ecomesure, Saclay, France). Based on the outcome of the experiments, the MCE filter and mini particle sampler were chosen for the final set-up.
ICP-MS and STEM-EDX were used as characterization techniques to determine the concentration and chemical composition of particles caught on the filter and grid, respectively. Rinsing water used to remove deposited particles from the inhalation tube before and after sampling was collected and also analyzed by ICP-MS.
The set-up was validated using face masks spiked with TiO_2 and Ag nanoparticles (positive controls). It was demonstrated that particles released from the mask during breathing simulation could be detected in the rinsing water and on the filter. Some nanoparticles were also detected on the grids. However, contamination in the negative control and negative results in certain positive controls made interpretation of the results difficult. Therefore, for future experiments, a more extended validation of the set-up, making sure released particles are effectively detected and distinguished from contamination, should be performed.

Results of simulated breathing experiments

Three commercial masks, AgMask-03, AgMask-08 and AgMask-15, were tested in triplicate to determine possible releases of TiO_2 and silver (**Error! Reference source not found.**).
The observed variation in the amount of TiO_2 detected on the filters was high for the different replicates of all masks. Also in the experiment reported in Table 1, the result of the blank was higher than that of two earlier experiments (0.24 and 0.15 µg TiO_2) and also higher than the amounts of TiO_2 measured for all masks. No definite conclusion regarding TiO_2 release during the breathing cycle could be drawn due this high variation. No increased TiO_2 concentration compared to the blank was found in the rinsing water for the three types of masks and no TiO_2 particles were detected on the grids that could be traced back to the TiO_2 particles found on/in the mask.
No increased silver concentrations above the detection limit were found on the filters or the rinsing water for AgMask-03 and AgMask-15. In the rinsing water of one mask AgMask-08, a silver concentration just above the detection limit was measured. In addition, the silver concentration on the filters showed increased values compared to the filter blank (5 times higher) for all three of the tested AgMask-08

samples. Although no silver particles were detected on the grid, the results of the rinsing water and filter show that silver was released from AgMask-08.

Adding the numbers of the total silver recovered in the rinsing water of the third replicate and the average amount of silver obtained from the filter analysis divided by the inhaled volume of 9 m^3 resulted in an inhaled silver concentration of 0.004 $\mu g/m^3$. The measured silver values can be indicatively compared with the Derived-No-Effect-Level (DNEL) for silver defined for the general population listed in the ECHA database[32]. The DNEL for systemic effects after long-term exposure to nano-silver by inhalation is 2.0 $\mu g/m^3$. This is notably higher than the measured value, but results must be interpreted with caution because, in the absence of suitable reference materials, no information is available on the sampling efficiency: the recovery rate is unknown.

Table 1 Total titanium dioxide and silver (µg) recovered in the trachea tube rinsing water and on the filters after breathing simulation with different masks.

Face mask	TiO_2 rinsing water µg	TiO_2 MCE filter µg	Ag Rinsing water µg	Ag MCE filter µg
Blank, no mask	0.02	0.38	<0.007*	0.005
AgMask-03 1	0.02	0.09	<0.009*	0.004
AgMask-03 2	0.01	0.24	<0.008*	0.003
AgMask-03 3	0.02	0.07	<0.007*	0.003
AgMask-08 1	0.02	0.12	<0.009*	0.027
AgMask-08 2	0.02	0.08	<0.007*	0.020
AgMask-08 3	0.02	0.12	0.008	0.029
AgMask-15 1	0.02	0.08	<0.008*	0.002
AgMask-15 2	0.02	0.08	<0.007*	0.002
AgMask-15 3	0.02	0.32	<0.007*	0.002
AgMask-15 4	0.02	0.19	<0.007*	0.002

* Lower than detection limit of 0.1 µg/l

Conclusions

Drawing final conclusions related to particle release and associated risks mimicking real-life conditions is difficult considering the current state-of-the-art of the experimental set-up. Although the tests were executed in a HEPA-filtered, particle-free test chamber, the ubiquitous presence of titanium compounds nevertheless seems to hamper the identification of the possible contribution of TiO2 released from masks in the current experimental set-up. Even though no significant TiO_2 release from three different commercial masks could be measured, the lack of reproducibility both for blank runs and test masks requires a further validation of the set-up. For silver, only AgMask-08, which contains a silver coating on the fibres, showed a detectable release of silver. Even though the detected amounts on the filter were still in the range of the detection limit, knowledge on the sampling efficiency of our set-up based on reference control samples is necessary to conclude on absolute values of release. The size of released silver particles could not be determined, since the released amounts, and certainly the fraction of released particles captured on the EM-grids, were presumably too low to be traced back.

RELEASE OF TiO_2 PARTICLES AND SILVER-BASED BIOCIDES FROM FACE MASKS IN LEACHING EXPERIMENTS

Introduction

Silver nanoparticles have been used in fabrics for its antibacterial properties, while TiO_2 particles have been incorporated in textiles to provide UV-protection, self-cleaning, hydrophobicity and also antimicrobial properties[23,33]. Because of the apparent positive effects, textiles functionalized with nanoparticles were quickly used for the production of face masks during the COVID-19 pandemic[1]. Antibacterial and antiviral properties were the main claims of face masks toward coronavirus disease prevention, though in many cases such claims were not supported[34].

Health concerns regarding the daily use of textiles functionalized with nanoparticles are sustained in a number of studies that showed their potential release during experimental conditions that simulate normal wearing and/or washing[35,36]. For example, Quadros et al. investigated the release of silver from different children's products (including textiles) exposed to artificially prepared body fluids (i.e. saliva, sweat, urine) and showed that sweat and urine yielded the highest amount of released silver (38% of total). The explanation was an enhanced Ag dissolution due to the high salt concentrations of these fluids[36]. Rovira et al. studied the migration of silver and titanium (and other trace elements) in artificial sweat from different clothing items. In their study, titanium released in only one of the samples in detectable concentrations (7% of total titanium content), whereas silver migration rates ranged from 19% up to 25%[37]. In the above mentioned studies, migration experiments to evaluate leaching of Ag and Ti, were conducted with textile materials that are in close contact with the skin, so dermal exposure was considered when a risk assessment was performed.

To date, there is no standard method to evaluate leaching of inorganic nanoparticles from functionalized textiles that are in contact with the skin. Variations to the method proposed by von Goetz et al., which includes chemical stress (simulants) and physical stresses (abrasion)[35], have been used to evaluate release of chemical substances, including nanoparticles, from textiles. The advantage of such leaching methods is that they are cheap and easy to conduct under normal laboratory conditions.

The research questions addressed in the AgMask and TiO_2Mask projects were to determine (1) if silver-based biocides and TiO_2 particles that are present in the fabrics of face masks, are released under normal and/or extreme usage conditions, and (2) if there is release, to what extent. Such information could contribute to the risk assessment and regulatory control. As shown in the previous sections, conventional abrasion methods applied to textiles and experiments simulating normal breathing were conducted to evaluate release of silver and TiO_2 particles from face masks. Since in none of the cases conclusive results could be obtained, leaching experiments were conducted as an alternative and more simple method to evaluate the release of silver biocides and TiO_2 particles present in face masks. It is not the intention to translate the leaching data into the risk analysis, but the leaching data can be used to refine the preliminary risk assessment presented in the intermediate reports of the AgMask and TiO_2Mask projects.

Materials and Methods

SELECTION OF FACE MASKS FOR MIGRATION EXPERIMENTS

Migration experiments were conducted with face masks that were intended to be worn by the general public. The face masks were commercially available in Belgium and on the EU market and were purchased in 2019 for the AgMask and TiO_2Mask COVID-19 projects. For the migration experiments, 10 different types of face masks were selected based on availability and on the measured total content of TiO_2 and Ag per mask as reported by Mast et al.[16,18]. The selection aimed to include a range of TiO_2 and Ag levels in the face masks, i.e. non detected, low, medium and high (Table 2).

Table 2 Overview of the selected face masks used in the leaching experiments

Face mask	Ag[a] (µg/mask)	TiO_2[a] (µg/mask)	Type of mask	Form of Ag biocide
AgMask-05	N.D.	791	Single-use	
AgMask-11	N.D.	11	Single-use	
AgMask-20	6.5	2298	Reusable	Ag^+ ions
AgMask-12	7.3	8175	Single-use	Ag^+ ions
AgMask-13	13	12196	Reusable	Ag^+ ions
AgMask-03	37	30752	Reusable	Ag^+ ions, NPs and large Ag^0 particles
AgMask-14	87	29	Reusable	Ag^+ ions
AgMask-15	165	152345	Reusable	Ag^0 NPs
AgMask-18	176	19146	Reusable	Ag^+ ions
AgMask-08	235044	12297	Reusable	Ag^+ ions, Ag^0 NPs, Ag^0 coating

N.D.: not detected

[a]Total Ag and TiO_2 in the face mask was determined by ICP-MS and ICP-OES after microwave digestion in concentrated acid, from published data[17, 19, 16,18].

SELECTION OF ARTIFICIAL ACID SWEAT AS LEACHING SOLUTION TO ASSESS MIGRATION OF SILVER AND TITANIUM FROM FACE MASKS IN MIGRATION EXPERIMENTS

Because the release of substances from textiles in leaching experiments depends on the properties of the leaching solution, including the pH and salinity, leaching experiments typically choose to use an artificial physiological buffer as leaching solution. In the absence of a standardized protocol that simulates the conditions of the liquid in saturated breathed air, our experiments were aligned with previous studies that investigated metal leaching from textiles in close contact with the skin, using artificial human sweat solutions in order to simulate some type of chemical stress that would stimulate the release of metals from the textiles[35,38–42]. Although there is no unique protocol to prepare artificial human sweat solutions, a number of published scientific works that evaluated the release of metals from textiles followed the protocol described in the ISO procedure 105-E04 for 'color fastness to perspiration'[43]. In the ISO protocol the pH of the artificial sweat solution can be adjusted to pH 5.5 or 8.0 by adding a respective amount of sodium hydroxide (NaOH). To test the migration of Ti and Ag from the face masks in this work the artificial sweat was adjusted to pH 5.5, considering that the pH of human sweat is slightly acidic with reported median pH 5.3[44]. The acid artificial sweat solution was prepared by dissolving 5 g of sodium chloride (NaCl), 2.2 g of sodium dihydrogen orthophosphate dihydrate

($NaH_2PO_4 \cdot 2H_2O$) and 0.5 g of L-histidine monohydrochloride monohydrate ($C_6H_9O_2N_3 \cdot HCl \cdot H_2O$) in 1000 mL of bi-distilled water. The solution was brought to pH 5.5 (±0.2) with 0.1 M NaOH.

For the migration experiment a piece of face mask (6 cm x 6 cm) was cut, accurately weighed and placed in a 50 mL polypropylene tube. Then, 20 mL of freshly prepared artificial acid sweat (pH 5.5) was heated to 37°C and added to each tube containing the face mask sample. The tubes were then screw-tightened with a lid and agitated at a speed of approximately 57 rpm in an overhead shaker that was placed inside a pre-heated (37°C) oven. After a contact time of either 1, 4, 8 or 24 h, the piece of face mask was removed from the tube. Next, 1 mL of the supernatant leachate solution was subsampled and acidified to 4% v/v HNO_3 using concentrated Suprapur® nitric acid (HNO_3 65%). Samples were analysed for Ag and Ti by inductively coupled plasma mass spectrometry (ICP-MS/MS, Agilent 8800) without further processing. Control blank samples (without face mask) and spiked blank samples for Ag 100 µg/L and Ti 10 µg/L were also included in the experiment. The migration experiment was conducted in duplicates. For face masks that were composed of layers with distinctive fabrics or element content (i.e. AgMask-08), the leaching was conducted on each separate layer.

The ICP-MS analysis for Ag was performed in helium (He) collision cell mode monitoring ^{107}Ag isotope and for Ti the ^{48}Ti isotope was measured in mass-shift mode (m/z 64) using a mixture of oxygen (O_2) and hydrogen (H_2) cell gases to resolve isobaric and polyatomic interferences, which can challenge the analysis of ^{48}Ti by ICP-MS. Matrix matched calibration standards were prepared using certified single element standard solutions (1000 mg/L). All solutions were acidified with concentrated Suprapur® nitric acid (HNO_3 65%). An overview of the methodology used for the leaching experiments is shown in Figure 3.

Estimation of the LOQ of the method was based on the analysis of 20 consecutive matrix blank samples that were prepared independently. The calculation of the LOQ was done according to Wenzl et al.[45]. The calculated limit of quantification (LOQ) for Ag was 0.015 µg Ag/L and for Ti 0.16 µg Ti/L. Measurements below the calculated LOQ were reported as <LOQ.

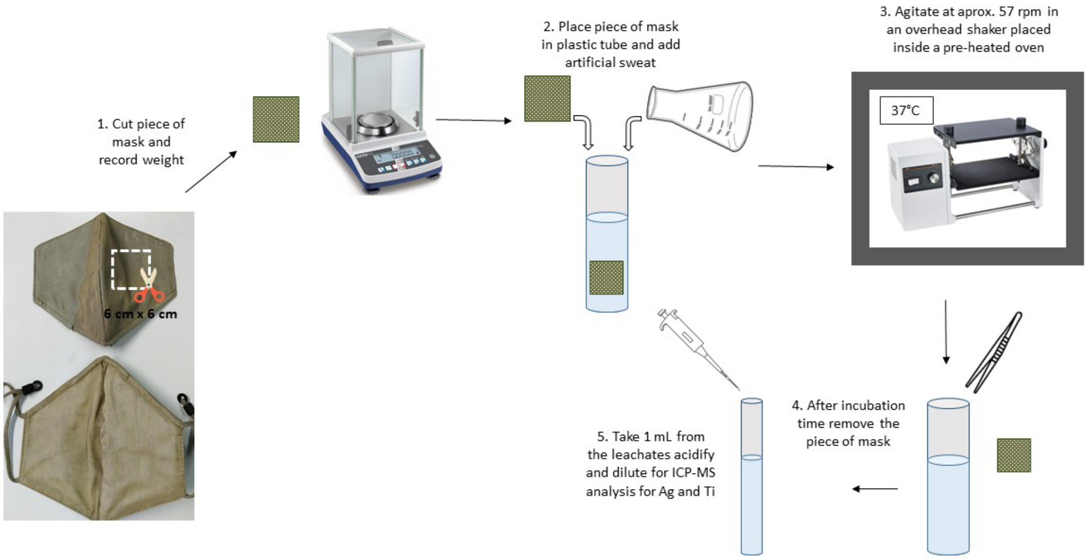

Figure 3 Overview of the methodology used for the leaching experiments.

Results and Discussion

RELEASE OF SILVER IN LEACHING EXPERIMENTS

No Ag was detected in the leachates of face masks AgMask-05 and AgMask-11 after the different equilibration times (data not shown). These results were expected since the face masks were included in the experiment as negative controls (i.e. face masks contained no Ag). Additionally, no Ag was detected in the control blank samples (absence of face mask).

Silver was released into artificial acid sweat in concentrations above the LOQ in the following face masks: AgMask-08, AgMask-15, AgMask-18 and AgMask-20 (Table 3). In general, the highest amounts of Ag that leached out into acid sweat were observed in the external and internal layers of AgMask-08 with over 20 µg Ag/g sample. The central layer of AgMask-08, which consisted of 4 layers of non-woven fabric also released Ag, although at much lower concentrations of < 2 µg Ag/g sample. As reported previously by Mast et al.[16] the external and internal layers of AgMask-08 contained also the highest total content of Ag per layer with more than 110 mg Ag, while the central layer contained 0.2 mg Ag. It appears that the difference in the degree of leaching in the external and internal layer compared to the central layer can be related to their initial total Ag content. Electron microscopy analysis showed that the woven fibres of the external and internal layers were coated with Ag^0 nanoparticles and that the coating was damaged or incomplete in many areas[16]. The presence of Ag in the central layer can be explained by the observed damage of the coating, resulting in detached Ag^0 nanoparticles in the central layer.

Comparing the release of Ag into the sweat solution under the different contact times, no significant differences (*P* value > 0.05) were observed in the amount of Ag released during time in any of the layers. This suggests that an extended exposure of the fabrics to sweat through wearing does not necessarily result in more release of Ag. Similar Ag leaching kinetic behaviour has been reported previously by Quadros et al. 2013 [36] and von Goetz et al. 2013 [35] for Ag-containing textiles in contact with artificial sweat. This information of the leaching kinetics is useful to refine the safety assessment of re-usable face masks when taking re-use into consideration. These observations contradict the hypothesis that silver-based biocides are gradually or linearly released from face masks over time.

Face mask AgMask-15 consisted of an external and internal layer of polyamide containing about 80 µg of Ag each. Since both layers were identical in material composition and Ag content, the layers were not separated for the leaching experiments. As shown in Table 3, the release of Ag was < 1 µg Ag/g sample and the amount of Ag that released to the acid sweat reached a maximum after 1 h remaining relatively constant over the following leaching times (no significant differences *P* value > 0.05 for release of Ag at different contact times). Mast et al. showed that in AgMask-15, Ag was present as nanoparticles which were in large part encapsulated in the polymer fibres arguing that most of this nanoparticulate Ag would be unavailable for release[16]. The lower release of Ag per g of material in AgMask-15 confirms this statement.

Face mask AgMask-18 was made of two identical layers containing polyester, polyamide and elastane. The leaching experiment was however conducted on separate layers (external, internal) since the Ag content slightly differed in both layers (66 µg Ag external layer vs. 110 µg Ag internal layer)[16]. As shown in Table 3, the release of Ag increased from 8 µg Ag/g up to 13 µg Ag/g, but the high variation did not allow to show a significant difference (*P*-level > 0.05) between the two contact times of 1 h or 8 h.

Face mask AgMask-20 was composed of three different layers, the external and internal layer were made of non-woven fabric and the central layer of nanofibre membrane. The central layer contained the highest amount of Ag (5.4 µg) while the external and internal layer contained 0.5 µg Ag and 1.2 µg Ag, respectively[16]. As shown in Table 3, Ag released in detectable concentrations only from the central layer and the amount of Ag released after 8 h contact time was about three times higher compared to 1 h contact time.

Across all face masks the average amount of Ag released per mask followed the decreasing order: AgMask-08 ~ AgMask-18 > AgMask-15 >> AgMask-20 (Table 3). Face mask AgMask-08 and AgMask-18 had similar levels of total Ag release per mask, (63 µg Ag/mask average of 4 leaching times for AgMask-08 and 64 µg Ag/mask average of 2 leaching time for AgMask-18), despite AgMask-08 having more than thousand times higher total Ag content. When the release is expressed as percent of total Ag content, AgMask-08 yielded the lowest percent of released Ag (0.03%) compared to AgMask-18 (36%). In addition, despite AgMask-15 and AgMask-18 having more or less similar total Ag content (165 vs 176 µg Ag) the percent of Ag released was 6.5 times higher in AgMask-18 than AgMask-15.
The differences can be related to the form of Ag biocide present in the fabric and also on the functionalization technology. For example, Wagener et al. reported between 8 to 75% of total Ag content released into artificial sweat in textiles where Ag was added as a surface coating, whereas either no release or < 8% of total Ag content was released in textiles where Ag was embedded as a nanocomposite in the fabric[40]. Furthermore the authors concluded that the release of Ag predominantly occurred as dissolved Ag. In the present study, for AgMask-18, Ag is present as Ag^+ ions, in AgMask-15, Ag is added as Ag nanoparticles and AgMask-08 contained Ag^+ ions and also Ag nanoparticles in the outside and inside of the fibres added as a coating[16].

Table 3 Amount of silver released into artificial acid sweat for face masks: AgMask-08, AgMask-15, AgMask-18 and AgMask-20

Face mask	Layer	Contact time (h)	Amount of Ag leached (µg Ag/g sample[a])	Ag leached per mask (µg Ag/mask)	Ag leached (% of total Ag content)
AgMask08	External Central Internal	1	22 ± 1.1 1.3 ± 0.5 23 ± 4.1	61	0.03
	External Central Internal	4	27 ± 2.3 1.2 ± 0.3 23 ± 1.3	66	0.03
	External Central Internal	8	24 ± 0.8 1.6 ± 0.02 24 ± 2.5	65	0.03
	External Central Internal	24	21 ± 0.5 1.8 ± 0.5 22 ± 0.8	59	0.03
AgMask15	All	1	0.94 ± 0.02	9.3	6
	All	4	0.88 ± 0.02	8.8	5
	All	8	0.97 ± 0.16	9.6	6
	All	24	0.87 ± 0.10	8.6	5
AgMask18	External Internal	1	8.8 ± 0.31 8.2 ± 0.92	51	29
	External Internal	8	12.3 ± 5.8 13.0 ± 5.0	76	43
AgMask20	External Central Internal	1	<LOQ 4.9 ± 2.8 <LOQ	0.09	1
	External Central Internal	8	<LOQ 16 ± 3.2 <LOQ	0.3	5

[a] Average of duplicates ± standard deviation

Finally, in four face masks AgMask-03, AgMask-12, AgMask-13, AgMask-14 no Ag was detected in the artificial sweat at any of the contact times (data not shown). AgMask-03 contained three distinctive layers (external, central and internal) and Ag was only found in the external layer[16]. *In situ* electron microscopy analysis showed that at least a fraction of the total Ag was present as Ag nanoparticles which were observed at the surface or close to the cotton fibres. In face mask AgMask-13, Ag was detected in the external and internal layers while in AgMask-14 Ag was detected in the central and internal layer. No Ag particles were observed by electron microscopy in these face masks so Ag was mainly present as Ag^{+} ions. In these face masks it can be speculated that Ag was not detected in the artificial sweat because the amount of released Ag was below the limit of detection, the coating of Ag was unevenly distributed in the face mask or for the specific case of AgMask-03, Ag was strongly bound to polycationic polymers which were incorporated in the fibres and thus not easily leachable.

RELEASE OF TIO_2 IN LEACHING EXPERIMENTS

All investigated face masks contained Ti as TiO_2 particles with contents ranging from 11 to 152345 µg TiO_2 /mask (Table 2). From the ten face masks investigated, only in mask AgMask-18 Ti was released into artificial sweat in measurable amounts (Table 4). The total percent release of Ti per mask was 0.35% (average of two leaching times). In the previous work, Verleysen et al. demonstrated the presence of TiO_2 particles in polyester and polyamide fibres with most of the particles located inside the fibres, although also some particles can be present at the surface of the fibre[19]. Presumably in this particular mask, TiO_2 particles at the surface were the ones that released during the leaching experiment since, supporting on the considerations of Franz et al.[46], larger particles firmly incorporated into the fibre do not leach out. No significant differences were observed between leaching of Ti at the two contact times.

In general the results from this work agree with other published studies that investigated the migration of TiO_2 particles from textiles in artificial solutions. For example, von Goetz et al. reported release of particulate TiO_2 in artificial sweat from a textile composed of polyester and wool [35]. No dissolved Ti was detected in the leachate solutions after ultrafiltration. The authors of the study speculated that the migration of TiO_2 particles in this particulate fabric was because they were added as a coating and hence not embedded in the fabric.
More and in depth research is needed to increase the understanding of which mechanisms and parameters, including the quality of the face masks, the type of fabric, and the localisation, influence the release of TiO_2 particles and Ag-based biocides and subsequent exposure.

Table 4 Amount of titanium released into artificial acid sweat for face mask AgMask-18

Face mask	Layer	Contact time (h)	Amount of Ti leached (µg Ti/g sample[a])	Ti leached per mask (µg Ti/mask)	Ti leached (% of total Ti content)
AgMask18	External	1	5.5 ± 1.6	34	0.3
	Internal		5.7 ± 1.8		
	External	8	8.4 ± 6.0	47	0.4
	Internal		7.1 ± 5.1		

[a] Average of duplicates ± standard deviation

REFINEMENT OF THE PRELIMINARY RISK ASSESSMENT WITH LEACHING DATA

The conditions in leaching experiments can be considered to be more stringent than those occurring during normal use of face masks. In such experiments, face mask samples are completely immersed in a solution of acidic pH and high salt concentration (artificial acid sweat) and they are subjected to physical stress since the tubes containing the samples, were shaken at a moderate speed in an end-over-end shaker. Hence, leaching experiments can be applied as an alternative and relatively cheap method to evaluate the potential release of selected chemicals from face masks, and as a higher tier method to refine the risk assessment for the face masks that are not safe-by-design.

In the intermediate reports of the AgMask and TiO_2Mask projects a conservative preliminary risk assessment was conducted for the face masks by comparing the total amount of silver biocide or TiO_2 per mask to their respective acceptable exposure level per mask (AEL_{mask}). The estimated AEL_{mask} for Ag in generic form was 25 µg, for Ag nanoparticles 2.25 µg, while for TiO_2 AEL_{mask} was 3.6 µg. For details on how the different AEL_{mask} for Ag and TiO_2 were determined, the reader is referred to the Intermediate Reports for AgMask and TiO_2Mask projects[16,18]. Masks for which total amounts of Ag or TiO_2 were below the AEL_{mask} could be considered as intrinsically safe, while masks with total amounts above the AEL_{mask} required a more refined risk evaluation.

The data obtained from the leaching experiments were used to refine the preliminary risk assessment. For that, the threshold values (AEL_{mask}) were compared with the total leachable Ag per mask from the leaching experiments. As can be seen in Table 5, for AgMask-08 and AgMask-18 the total leachable Ag per mask is higher than the AEL_{mask}, hence these face masks need further refined safety assessment. However, the ratios are much lower than those calculated with the total Ag content in the mask. This approach is still conservative because the conditions of the leaching experiments are harsh compared to the actual wearing conditions but they are more refined than simply using the total amount present in the face mask. For AgMask-15 the total leachable Ag is below the AEL_{mask} (using the threshold limit for generic Ag), which implies that this mask can be considered safe based on this conservative approach. However, when the ratio is calculated using the threshold for Ag^0 NP it shows that AgMask-15 would need a more refined assessment. For masks AgMask-03 and AgMask-14 no ratio could be calculated since in these face masks Ag was not detected in the acid sweat leachates, thus indicating that these masks can be considered safe based on this conservative approach.

Following the same approach as for Ag, the risk evaluation could be refined for TiO_2 using the leaching data (Table 5). Titanium was detected only in the leachate of mask AgMask-18 and the total amount of Ti released after 8 h was 47 µg Ti/mask (78 µg TiO_2/mask). This amount is 22 times higher than the threshold AEL_{mask} value (3.6 µg). Hence, we cannot conclude on the safety of this mask.

Table 5 Exceedance ratios for silver and TiO_2 calculated using the total silver and TiO_2 content per mask or the total leachable silver and total leachable TiO_2 per mask divided by their respective limit value (25 µg Ag in generic form, 2.25 µg Ag nanoparticles or 3.6 µg TiO_2)

Face Mask	Times total amount of Ag exceeds AEL_{mask}[a]	Times total amount of leachable Ag exceeds AEL_{mask}[b]	Times total amount of TiO_2 exceeds AEL_{mask}[c]	Times total amount of leachable TiO_2 exceeds AEL_{mask}[d]
AgMask-03	1.5 / 16.4	N/A	8542	N/A
AgMask-08	9402 / 104464	2.6 / 29	3416	N/A
AgMask-14	3.5	N/A	8	N/A
AgMask-15	6.6 / 73	0.4 / 4.3	42318	N/A
AgMask-18	7.0	3	5318	22
AgMask-20	0.3	0.01	638	N/A

[a] Ratio of total Ag content per mask by limit value. The value before the slash is calculated using the generic threshold limit for Ag and the value after the slash is calculated using the threshold limit for Ag nanoparticles.
[b] Ratio of total leachable Ag per mask by limit value. The value before the slash is calculated using the generic threshold limit for Ag and the value after the slash is calculated using the threshold limit for Ag nanoparticles.
[c] Ratio of total TiO_2 content per mask by limit value.
[d] Ratio of total leachable TiO_2 per mask by limit value.
N/A: Denotes that ratios were not calculated as Ag or Ti were not detected in the leachates.

CONCLUSIONS

From the face masks investigated, the highest release of silver per mask was observed in masks AgMask-08, AgMask-15 and AgMask-18. When expressing the release of Ag as percentage of total content in the mask, AgMask-18 leached up to 43% of total silver (36% average of two leaching times), whereas for AgMask-15 and AgMask-08 the leached silver represented about 5% and <1% of the total amount, respectively. The leaching data of titanium showed that despite TiO_2 being detected in all face masks, only in one mask titanium was measured in detectable concentrations in artificial sweat (0.35%). The plausible explanation of the limited release of titanium can be related to the localization of the large majority of the TiO_2 particles in the fibres.

In this work, a simple method was used to estimate the release of silver and titanium from face masks. The applied leaching conditions, combining the application of chemical stress (i.e. acidic pH of the artificial sweat solution) and physical stress (i.e. end-over-end shaking), can be considered more stringent than those occurring during normal face mask wearing. The leaching experiments were examined as a “proxy approach”, which is also a conservative one, in order to bypass the technical challenges encountered by the lack of methodology to determine and quantify release of Ag-biocides and TiO_2 particles from face masks in conditions that mimic real wearing. With these considerations face masks AgMask-08, AgMask-15 and AgMask-18 failed to pass this proxy test. We acknowledge that leaching experiments would require further development and standardisation in order to be applied as an alternative and relatively cheap method to evaluate the potential release of selected chemicals. This standardisation, and further research of the mechanisms and parameters that influence the release of TiO_2 particles and Ag-based biocides in leaching experiments can underpin the application of leaching experiments as a higher tier method to refine the risk assessment when face masks are not safe-by-design, and possibly as a more efficient "safe by design" approach, reducing the need for full scale, *post-hoc* risk assessment of every possible product.

EXTRACTING TiO_2 PARTICLES FROM MASKS FOR ACCURATE SIZE MEASUREMENT

Introduction

The possible effects of inhaled nanoparticles on the human body are highly dependent on their size and shape properties[47]. Therefore, to assess the risks associated with TiO_2 that is found in masks, an accurate size measurement of the TiO_2 particles embedded within the fibres is crucial. Within a regulatory context, especially the size of the constituent particles is an important measurement which determines whether a material is classified as nanomaterial or not[48].

In the first part of the AgMask project, *in situ* TEM imaging of cross-sections of mask fibres showed that TiO_2 is typically embedded as aggregates/agglomerates[19]. While this approach allows the localization of the particles, there are some drawbacks related to the size measurement of the aggregates/agglomerates and especially the constituent particles. Since the particles are fixed in a matrix, i.e. the fibres, they cannot be manipulated to try to separate aggregates/agglomerates into constituent particles. As a result, we have to rely on manual measurement of the constituent particle size based on a limited data set of high-magnification images, which is a time-consuming and relatively less precise process. In addition, the sample preparation, which involves embedding pieces of masks in epoxy and cutting ultra-thin slices using diamond knives, is tedious, time-consuming and costly. Due to these factors, imaging of the particles embedded in the fibres is not an ideal method to apply for control of face masks. Therefore, a new methodology, aiming at a more accurate and cost-efficient particle size measurement of TiO_2 was developed. The protocol is based on dissolving the fibres of the masks to separate the TiO_2 from their matrix, dispersing and stabilizing them in suspension and bringing them on a TEM grid for analysis.

Digestion protocol to extract TiO_2 particles

After a literature search on solubility of different fabrics, specific chemical solvents were tested on mask layers of different composition (polyester, polyamide, polyethylene, or a mix of different ones). For purely polyester and polyamide mask layers, a working digestion protocol was developed, based on basic and acid solvents, namely potassium hydroxide (KOH) and hydrochloric acid (HCl), respectively. More aggressive solvents and protocols at elevated temperatures were tested to dissolve the more resistant polypropylene fabrics, but were all unsuccessful. Either the fabric did not dissolve, or applying a very harsh protocol, both the fabric and TiO_2 particles dissolved, not allowing the purpose of the experiment. For mixes of fabrics, first tests have been done using different solvents. Up to now, the best result gives partial digestion but not enough TiO_2 particles were retrieved to make a quantitative analysis possible.

After digestion of the fibres, TiO_2 particles are extracted by centrifugation and dispersed in solution. A sonication step is applied to try to break up tightly bound aggregates. By casting a drop of the sample suspension on a positively charged TEM grid, TiO_2 particles attach to the grid, which can be analysed by electron microscopy.

Size measurement by TEM

For polyester and polyamide mask layers, the digestion and specimen preparation protocol resulted in a concentration of particles on the grid suitable for a quantitative analysis of the particle properties including their size. As example, Figure 4A shows a representative TEM micrograph of the TiO_2 particles (dark on a bright background) extracted from the polyester external layer of AgMask-03. Both single particles and aggregated and agglomerated particles can be found back. For comparison, a STEM image (inverted contrast) of TiO_2 particles inside the polyester fibre (oval shape) of AgMask-03 is added in Figure 4B. For a quantitative analysis of the aggregate/agglomerate and constituent particle size, the extracted particles in image A are more suitable, because of the following points:

- The particles are less aggregated, which makes it easier to identify the constituent particles within larger ensembles.
- The particles are more concentrated:
  - ➔ Imaging can be done efficiently at higher magnification, still capturing multiple particles in one image, to increase the resolution for a more accurate size measurement.
  - ➔ Increased statistics for the size measurement can be achieved in the same imaging time, increasing the precision of the measurement of the median value of the distribution.

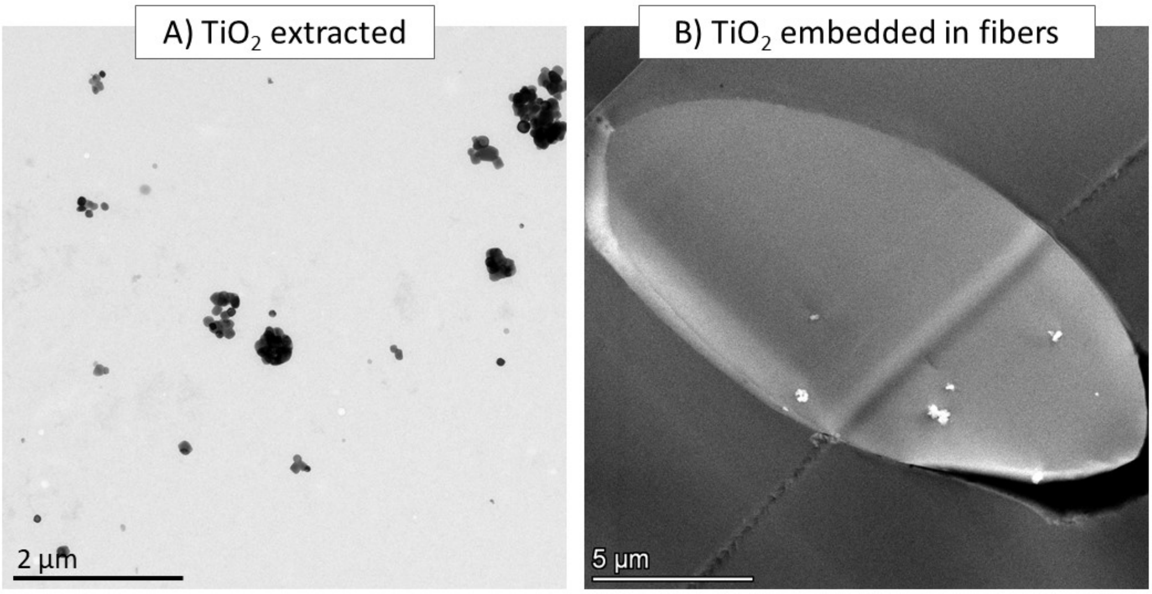

Figure 4: Representative TEM and STEM images used for quantitative analysis of TiO_2 particles obtained by (A) applying the extraction method on AgMask-03, (B) applying the *in-situ* identification method on AgMask-03.

Using the image analysis software ImageJ[49], we can rely on a semi-automatic size measurement of the aggregates/agglomerates and the constituent particles. Aggregates and agglomerates are detected simply based on the contrast difference with the background. To identify constituent particles, a separation algorithm is applied subsequently. This algorithm occasionally gives problems for larger aggregates where particles are overlapping more, but it remains a considerable improvement compared to manual measurement, which was necessary for embedded TiO_2. Based on a series of multiple images, size distributions of the minimum Feret diameter (Fmin), representing the minimum external dimension of the particles, are obtained, as shown in Figure 5 for AgMask-03. The size of the aggregates/agglomerates varies between 20 and 900 nm. The constituent particle size varies between 20 and 230 nm. These measured values agree with the value for so-called textile grade TiO_2 widely applied in fabrics.

For all examined masks the median value of the Fmin size distribution of aggregates/agglomerates and constituent particles is shown in Figure 6, including a comparison with the values obtained from embedded TiO_2 particles during the first part of the project. The size values are consistent between all masks with no strong variations, except for the aggregate/agglomerate size in AgMask-22. For this specific sample, so little particles were attached to the grid that there was a bias towards spotting bigger agglomerates during imaging and a large error due to low statistics. Some inherent differences in the Fmin values as determined from extracted or embedded particles can be expected as a consequence of differences related to the applied method, involving different sample preparation, different imaging modes and manual versus semi-automatic particle analysis. The standard error is added on the median values, showing clearly a decrease in the error on the Fmin for constituent particles following our new methodology. The median values of the constituent particles are in all cases close to 100 nm, which means textile-grade TiO_2 should be assumed a nanomaterial, according to the European Commission's recommendation[48].

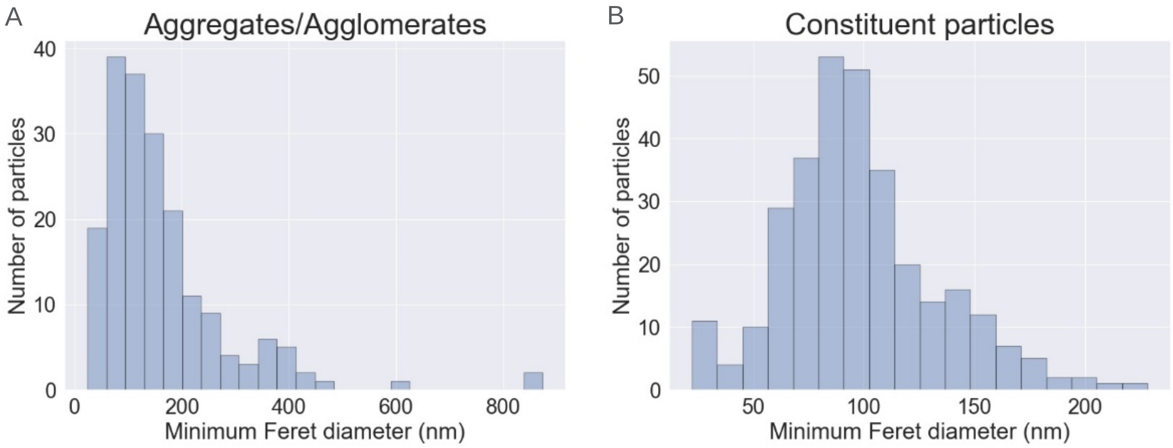

Figure 5: Histogram of the minimum Feret diameter of (A) TiO_2 aggregates and agglomerates and (B) constituent TiO_2 particles based on a series of 9 images of AgMask-03 that were semi-automatically analysed using ImageJ.

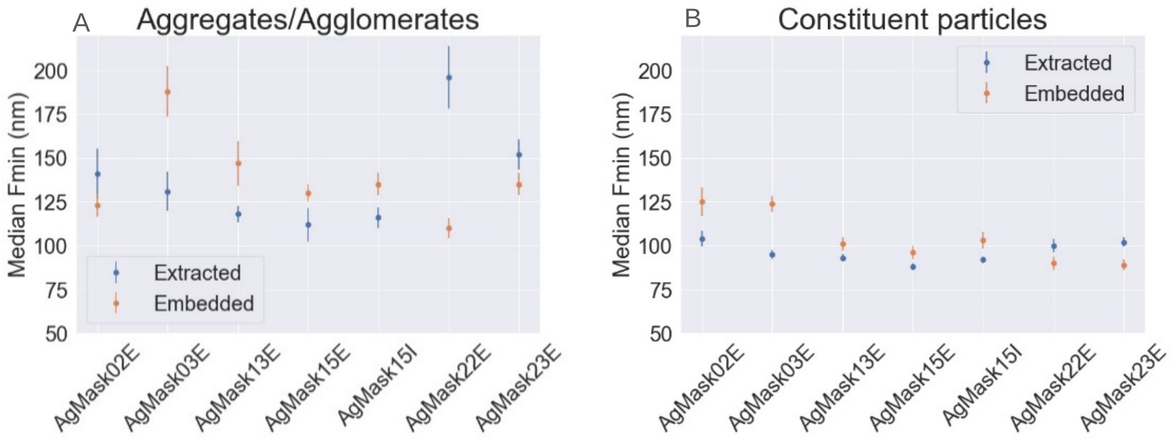

Figure 6: Median Fmin values with standard errors of TiO_2 (A) aggregates/agglomerates and (B) constituent particles in different mask layers, as measured on extracted particles after mask digestion or still embedded within the fibres. The E and I labels for the masks indicate external or internal fabric layers.

Conclusions

Summarizing, a methodology was developed for a more accurate and cost-efficient size measurement of TiO_2 nanoparticles in face masks or other textiles consisting of polyester or polyamide. In complement with imaging particles embedded within fibres, a full characterisation of nanoparticles in masks is achieved retrieving both information on localization of particles, releasable fraction and accurate size information. This method is useful for implementing regulatory control programs of face masks.

GENERAL DISCUSSION

Wearing face masks is an important and widely applied public health measure to control infectious agents[50]. Textile companies propose new solutions to the challenges associated with, for example, the COVID-19 pandemic, incorporating specific nanofibre, nanocomposite and nanoparticle technology into face masks[1], including the application of silver-based biocides[51]. Also, TiO_2 nanoparticles are widely applied in synthetic fibres during the production process, as a white colorant, as a matting agent, or to assure durability reducing polymer breakdown by ultraviolet light[52,53], and also as functionalizing agent to give various enhancing properties to the textiles[23].
The usefulness of face masks in the management of SARS-CoV-2 infections is unquestionable and the value of wearing face masks as a protection measure is undisputable[54]. Hence, we do not call for people to stop wearing face masks. However, the properties of silver-based biocides and of TiO_2 particles are not critical for the functioning of a face mask, and synthetic fibres suitable for face masks can be produced without TiO_2[22] as was also observed in the layers of several of the tested masks[19].
Therefore, it is important to assess whether the possible health risks associated with silver-based biocides and TiO_2 particles applied in face masks not outweigh their benefits. In this perspective, in depth research of (nano)technology applications in textiles, and specifically the added value of biocidal silver treatment of face masks, examined in the AgMask project, and the application of TiO_2 particles, examined in the TiO2Mask project, is important to avoid possible future consequences caused by a poorly regulated use. A first and important step is the development of detection and characterisation methods. Therefore, conventional methods were evaluated in the AgMask and TiO_2Mask projects, and several new methodologies were developed to characterise TiO_2 particles and silver-based biocides applied in face masks in detail and to obtain the data required for their safety assessment.

To evaluate the amounts and presence of silver-based biocides and of TiO_2 particles in face masks, an approach was set up that combines total silver and titanium measurement using ICP-MS or ICP-OES with *in situ* analysis of silver-based biocides and TiO_2 particles in ultra-thin sections of face masks using STEM and EDX.
The conventional method for digestion of the materials in matrices such as face masks typically requires very acidic conditions and the use of hydrofluoric acid[55]. To avoid this very hazardous acid, a digestion method at high temperature was validated[17,19,21]. The combination of this digestion method with ICP-MS and ICP-OES techniques allowed precise quantification of Ti and Ag covering the amounts present in the examined face masks. *In situ* analysis of silver-based biocides[17] and TiO_2 particles[19] in ultra-thin sections of face masks using STEM and EDX combines spatially resolved structural and chemical information to identify, localize and characterise properties of (nano)particles in textile fibres. A detailed description of the developed sample preparation, imaging and image analysis procedure is made publicly available[20]. This approach allows to identify and localize Ag and TiO_2 particles, and to differentiate between the type and appearance of the applied silver-biocide (ionic/nanoparticulate form, coating). The high resolution imaging capability of the STEM-EDX technique has proven to successfully identify silver particles of sizes down to 13 nm. Due to limited sampling, however, only a small part of the specimen can be examined, so that low numbers of nanoparticles (or non-homogeneities) might remain undetected. The proposed approach demonstrated the presence of TiO_2 particles in the synthetic fibres and varying amounts and different types of silver-based biocides in a selection of face masks offered on the Belgian market and intended to be worn by the general public. Following types of silver-based biocides were demonstrated: (i) Ag^+ ions, (ii) metallic Ag^0 NP distributed in the matrix of the fibres, (iii) Ag nanoparticles and large silver particles at the surface of, or close to cotton fibres in face masks containing polycationic polymers binding Ag^+ ions (Silvadur™ technology), (iv) as a coating consisting of metallic silver releasing Ag^+ ions, Ag^0 nanoparticles and large silver particles[17, 20].

Complementary to the *in situ* TEM imaging of TiO_2 particles in cross-sections of synthetic fibres, a new method was developed that allows dissolving polyamide and polyester fibres of face masks and other textiles, allowing to separate the TiO_2 particles from the polymeric matrix, so that they can be dispersed and stabilized in suspension and brought reproducibly on a TEM grid. Combined with quantitative image

analysis, this approach, relying on semi-automatic rather than on manual particle measurement and avoiding resin embedding and ultra-thin sectioning, allows a more accurate, cost-efficient and faster measurement of the size distribution of the constituent TiO_2 particles, as well as of their aggregates/agglomerates, than the methodology based on *in situ* TEM imaging of TiO_2 particles[19]. Efficient determination of the number-based size distribution of the constituent particles is critical for the implementation of the Biocidal Products Regulation (BPR), the REACH Regulation and the Regulation on the classification, labelling and packaging of substances and mixtures (CLP)[56]. These regulations have specific provisions for nanomaterials, which are defined according to the European Commission Recommendation on the definition of nanomaterials based on the number-based size distribution of constituent particles[48]. The proposed methodology can be adapted for measuring other types of (nano)particles and other types of synthetic fibres, provided that the extraction method effectively destroys the matrix without changing the properties of the particles of interest.
Measurements of the size and shape (near-spherical morphology) of the constituent TiO_2 particles and agglomerates in the examined face masks show that, overall, the physicochemical properties of the TiO_2 particles in face masks are in agreement with the specifications of so-called fibre-grade TiO_2 applied in other textiles[57,58] and are similar to those of the E171 food additive[59]. Applying the new, extraction-based measurement method, most of the applied TiO_2 qualified as nanomaterials according to the EC-definition[60], while all of the examined face masks consisting of synthetic fibres contained a notable fraction of nanoparticles (6% to 65%)[19], requesting an appropriate risk analysis.

The assessment of potential risks associated with the inhalation exposure of silver-based biocides and TiO_2 particles released from face masks during normal use, is constrained due to the lack of available data on such exposure or on release. Therefore, an approach comparing the measured Ag and TiO_2 content of the face mask with limit values, was applied to evaluate the safety of the face masks with a minimum of assumptions.
For silver, two limit values were considered, defined by the two forms of silver-based biocide, namely a generic one, where silver occurs as metal dust, fume, and soluble compounds, and a specific one, where silver occurs as nanoparticles. The National Institute for Occupational Safety and Health (NIOSH) has defined an occupational exposure limit (OEL) for both forms, i.e. 10 µg/m³ and 0.9 µg/m³ respectively [61]. Based on these OELs, limit values of respectively 25 µg and 2.25 µg, estimating the maximum amount of silver (µg) that can be released during a mask's use and inhaled without generating safety concerns, were calculated.
To evaluate whether the TiO_2 particles in face masks possibly present a health risk, the amount of TiO_2 at the surface of the textile fibres was estimated based on their amounts, their physicochemical properties and their localization in a selection of face masks.[19,20] This value was compared with 3.6 µg, the acceptable exposure limit for TiO_2 by inhalation, expressed per mask. This limit value was estimated using a threshold-based risk characterisation for subchronic exposure with an intensive use scenario of face masks by the general adult population. Detailed calculations of these limits are described in Mast et al. for silver[17] and in Verleysen et al. for TiO_2[19].

When the silver or the TiO_2 contents of a face mask exceeds a limit value in the presented approach, this does not imply a risk as such, but rather indicates the need for a more detailed assessment enabling to make a conclusion of the face mask's safety. On the other hand, if the content is lower than the limit values, it indicates that the mask can be considered intrinsically safe, independent of more detailed information on actual exposure. Hence, our approach is an efficient and transparent screening approach identifying safe face masks despite the scarcity of actual exposure information.

For all examined face masks, the amount of TiO_2 particles at the surface of the textile fibres notably exceeds the safety limit. This systematic exceedance indicates that by applying an approach relying on conservative assumptions while uncertainties regarding hazard and exposure remain, a definitive conclusion about their safety (intrinsically safe) cannot be made when face masks containing polyester, polyamide, thermo-bonded non-woven and bi-component fibres, are used intensively. Exceedance of the limit for reusable face masks is higher (87 to 1220 times) than for single-use masks (5 to 11 times), implying that for the reusable masks inhalation of only a very small percentage of the particles at the fibre surface may already pose a health risk. Reusable masks typically have higher TiO_2 amounts in the matrix, have a higher mass (more textile corresponds with more TiO_2), and have smaller mean fibre diameters than single-use masks.

More than half of the analysed face masks that contain detectable amounts of silver, contain levels well below the relevant limit values and can be considered intrinsically safe, independent of more detailed information on actual exposure. Several face masks contain, however, levels of silver which exceeded one or both of the limit values used in this study, and a definitive conclusion (intrinsically safe) regarding the safety of these could not be made.
It is clear that the approach chosen is conservative because several factors, discussed in detail by Verleysen et al.[19] and Mast et al.[17], were not considered (due to lack of information) but which are key drivers determining the total silver exposure during use. The main reason for this is, however, the absolute scarcity of specific or generic information about the TiO_2 and silver release from face masks.

The above-described approach has the advantage that it allows to identify face masks containing silver-based biocides and/or TiO_2 particles and that it allows to differentiate masks that can be considered intrinsically safe (safe-by-design) with a minimum of assumptions, from those that require a more extensive assessment. The latter requires information on the modalities of use (duration, frequency of change, washing, …) by the intended population, and on the release of silver-based biocides and/or TiO_2 particles from the face mask during use, which leads to inhalation exposure. In this perspective, a major limitation for the safety assessment of TiO_2 particles and silver-based biocides in face masks is the scarcity of quantitative (measured or modelled) information about their release during the use of face masks.
Some information is available on the silver release from silver-treated textiles during washing/dissolution [62–64] or abrasive conditions [65,66]. Release of TiO_2 particles was reported to be caused by exposure to a variety of stresses, fluids/solvents or other impulses during the different stages of the lifecycle of a mask [24,25]. Literature data are limited to studies specifically examining the release of TiO_2 particles from textiles during washing conditions. Such studies show that only small percentages of the total TiO_2 content are typically released[26–29]. This type of information is difficult to extrapolate into inhalation exposure information during face mask use. Evaluation of release of TiO_2 particles from face masks under different use conditions is largely lacking in literature.

Aiming for a quantitative estimation of this release, exploratory research was therefore initiated in the AgMask and TiO_2Mask projects to develop new methods for release measurement mimicking real-life use, and to evaluate existing methods for quality control of textile, including conventional abrasions test methods and leaching experiments.

The possible release of silver and TiO_2 in the form of ions and/or nanoparticles from face masks resulting in exposure by inhalation is an important knowledge gap. As an attempt to fill that gap and work towards a formal risk assessment, an experimental set-up was developed in collaboration with VITO to evaluate the release of silver and TiO_2 particles from selected face masks mimicking real-life breathing conditions. Overall, the measurement results, which are in the order of the limits of detection, suggest that the amounts of TiO_2 and Ag released from the examined face masks are low. No significant release of TiO_2 was observed, while only for the face mask AgMask-08, which contains a silver coating on the fibres, a detectable release of silver was demonstrated. However, the variation between replicate measurements was considerable, with a lack of reproducibility both for blank runs and within test masks. Also, the recovery rate of the measurement methods is unknown because there are no suitable reference materials available. Therefore, it remains difficult to draw definitive conclusions regarding to particle release and associated risks using the current experimental set-up. For future experiments, a more extended validation of the set-up, making sure released particles are effectively captured, detected and distinguished from contamination, should be performed.

Conventional rubbing and abrasion tests typically used to test the quality of textile materials were screened to evaluate their suitability for simulation of wear and tear of face masks and for their potential to induce release of TiO_2 nanoparticles, which can be measured. Four types of equipment, including a pilling box, a washing tester, a Crock meter and a Martindale tester, were evaluated to simulate release of TiO_2 from face masks as a result of friction (mimicking normal wear and tear). Although the abrasion clearly had an effect on the fibre structure, more sensitive detection methods were needed than the applied detection methods. Conventional SEM and infrared spectroscopy were, for the tested configurations, not suitable to make definite conclusions on release of TiO_2 particles. The need for reference fibre materials for such method development was underlined.

Since there are no methods that can estimate release of nanoparticles from textiles under dry conditions, an alternative method is to extract them by soaking the textiles in simulant solutions (i.e. artificial saliva, urine, sweat). In this project, leaching in artificial sweat was a simple method that allowed determining release of silver and TiO_2 particles from face masks. In these leaching experiments, face masks were exposed to more stringent conditions than would occur during breathing, so the release of silver and titanium can be considered as a worst-case. In general, the release of metals and nanoparticles from functionalized textiles depends on various factors that are related to the specific manufacturing process of the textile and to the external factors to which the textile can be subjected. For instance, it has been shown in leaching experiments with artificial sweat, that Ag-functionalized textiles release Ag but the extent of the release depends on the functionalization technology. In textiles to which Ag was added as coating on the surface of the fibres a high release of Ag was observed, whereas when Ag was embedded within the textile fibres (as nanocomposites) much lower release of Ag was demonstrated[40]. In the present work leaching of Ag was also higher in layers of face masks where Ag was added as coating (i.e. external and internal layers of AgMask-08) compared to those where Ag was fully integrated as a nanoparticle in the fibre (i.e. AgMask-15) when expressed in absolute values (i.e. µg Ag/mask). The leaching results thus aligned well with the microscopy results.

The actual reason/benefit of using Ag was not investigated in our study, but it is a relevant aspect when evaluating the presence of biocidal substances such as Ag in face masks for general use. Moreover, biocides are strictly regulated substances in the European Union[67] with only specific combinations of biocides and uses (product types) being allowed. The use of Ag as a biocide is in the EU only permitted for product type 9[68,69], i.e. for the preservation of fibrous or polymerised materials by the control of microbiological deterioration. The incorporation of a biocide in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties is not covered by product type 9, but rather product type 2[70]. Silver has a confirmed antimicrobial and antiviral activity[71] , but the increased protection and benefit of face masks treated with Ag(nanoparticles) against SARS-CoV-2 lacks substantiation[34]. Moreover, increased protection by masks thanks to silver-based biocides would rather fit a product type 2 use, which is not allowed in the EU. We could not retrieve claims related to the anticipated benefits of adding silver-based biocides to face masks as textile preservatives (product type 9). The use of biocidal substances, such as Ag (nanoparticles), which can have hazardous properties, in applications in close contact with the general public deserves careful justification.

CONCLUSIONS AND PERSPECTIVES

To evaluate the amounts and presence of silver-based biocides and of TiO_2 particles in face masks, an approach was set up that combines total silver and titanium measurement using ICP-MS or ICP-OES with *in situ* analysis of silver-based biocides and TiO_2 particles in ultra-thin sections of face masks using STEM and EDX. This approach allows to identify and localize Ag and TiO_2 particles, and to differentiate between the type and appearance of the applied silver biocide (ionic/nanoparticle form, coating).

Complementary, a new method was developed that allows dissolving polyamide and polyester fibres of face masks and other textiles, thus allowing to separate the TiO_2 particles from the polymeric matrix, and a more accurate, cost-efficient and faster TEM measurement of the size distribution of the constituent TiO_2 particles, which is important for the implementation of the BPR, REACH Regulation and CLP regulations. This new approach for accurate size measurement of the constituent TiO_2 particles in polyamide and polyester fibres of face masks can also be extended to accurately and precisely measure constituent particles of a different elemental composition in a large variety of consumer products, provided that continued research identifies the conditions to digest the matrix without changing the physicochemical properties of the particles of interest. Measuring the size distribution of the constituent particles is essential to define a material as nanomaterial according to the EC-definition, which is key in the European regulation on the application of nanotechnologies in chemicals and consumer products.
The assessment of potential risks associated with the inhalation exposure of silver-based biocides and TiO_2 particles released from face masks during normal use, is constrained due to the lack of available data on such exposure and release. Therefore, an approach comparing the measured Ag and TiO_2 content of the face mask with limit values, was applied to evaluate the safety of the face masks with a minimum of assumptions. This efficient and transparent screening approach allowed to differentiate masks that can be considered intrinsically safe from those that require a more refined assessment.

For all examined face masks, the amount of TiO_2 particles at the surface of the textile fibres notably exceeds the safety limit. This systematic exceedance indicates that applying an approach relying on conservative assumptions while uncertainties regarding hazard and exposure remain, does not allow for a definitive conclusion about the safety (intrinsic safety) of intensively used face masks containing polyester, polyamide, thermo-bonded non-woven and bi-component fibres that include these substances.
More than half of the analysed face masks that contain detectable amounts of silver, contain levels well below the relevant limit values and can be considered intrinsically safe, independent of the availability of more detailed information on actual exposure. Several face masks contain, however, levels of silver which exceeded one or both of the limit values used in this study, and a definitive conclusion about their (intrinsic) safety could not be drawn.

A conventional risk assessment, combining exposure with hazard assessment, could not be performed for silver-based biocides and for TiO_2 particles, because no methods are available to directly measure particle release and inhalation exposure from face masks when wearing them. Aiming for a quantitative estimation of this release, new methods were explored to measure particle release in conditions that mimic real-life use. In addition, existing methods for quality control of textile, including conventional abrasions test methods and leaching experiments, were also evaluated.
An experimental set-up simulating breathing was developed in collaboration with VITO to evaluate the release of silver and TiO_2 particles from selected face masks mimicking real-life breathing conditions. This state-of-the-art experimental set-up did not allow to establish definitive conclusions and requires further development and validation.
Conventional abrasion methods, typically applied to test the quality of textiles, were shown to have an effect on the fibre structure, but they were not suitable to draw a definitive conclusion on particle release.
Leaching experiments were evaluated to assess the release of silver-based biocides and TiO_2 particles from face masks. The tested method induced such chemical (i.e. acidic pH of the artificial sweat solution)

and physical stress (i.e. end-over-end shaking) that the applied leaching conditions can be considered as more stringent than those occurring during breathing. Leaching experiments were shown to be useful as an alternative and relatively cheap method to evaluate the potential release of selected chemicals from face masks, and as a higher tier method to refine the risk assessment if face masks are not safe-by-design.

Wearing face masks remains an important and widely applied public health measure to control infectious diseases. The many uncertainties on potentially health risk of nanoparticles in face masks must be put into perspective in view of the many uncertainties with regard to the degree of exposure and the conservative toxicological approach taken and does not outweigh the benefits of using face masks in pandemic control measures based on the current scientific knowledge.

Future research

In the course of this study, we identified several major challenges related to the analysis, characterisation and risk assessment of the application of Ag-based biocides and TiO_2 in face masks, which go beyond the scope of the study and require further research. Overall, scientific data are lacking on the presence of (nano)particles in face masks, their characteristics, the exposure which is related to consumer behaviour , and the general risks for the consumers and the environment. A “one-health-approach” is required to fill these knowledge gaps.

Product quality and its (regulatory) control

- The developed methodologies and expertise in this project can contribute to the implementation of regulatory standards regarding (quality) control and to phasing out or limiting the amount of Ag-based biocides and TiO_2 particles following the safe-by-design principle. These methods are however the result of exploratory research and require further optimization, standardisation and formal validation. In this perspective, it was underlined that reference materials required for method optimization and validation are completely missing and hence need to be developed.
- The scarcity of quantitative (measured or modelled) information about the release of TiO_2 particles and silver-based biocides from face masks while using them is a major limitation for the safety assessment of these substances. It needs further investigation because there is hardly any of such information available in literature.
- The mechanisms of release and the conditions that might provoke release of chemicals from textiles are not well known. The results of this study indicate that depending on their type (ionic, (nano)particles), chemical composition (Ag, TiO_2, Cu, …), localisation (surface, in fibres), and textile matrix (polyester, polyethylene, cotton, ...), their release and potential (health) effects can be very different. Because key information and insights allowing read across are still missing, a specific assessment needs to be undertaken on a case-by-case basis for the variety of (marketed) applications.
- Research is needed towards developing materials aiming to minimize the release of chemicals, including (nano)particles, from face masks and functional textiles, and establishing quality criteria for such materials from a safe-by-design perspective.

Risks Assessment and health impact

- The presented study on face masks for the general population is limited to a selection of face masks containing the main categories of silver-based biocides. To have a better view of the market, it should be extended to a larger, representative sample. The quality related to medical face masks and personal protective equipment masks were insufficiently covered.
- Possible health risks related to the professional use of face masks, such as the application of medical face masks and personal protective equipment masks, were not in the scope of this research project and require further attention.

- Information on the dermal and inhalation exposure of different types of biocides and (nano)particles in face masks, and the subsequent risks for vulnerable (sub)populations is lacking.
- Information regarding consumer behaviour, such as the frequency and intensity of use and the type of masks used, is required to quantitatively assess exposure of both the general public and professional users to chemicals released from consumer products such as face mask. This information is nevertheless completely lacking while essential for a quantitative risk assessment.
- The potential health impacts of wearing face masks containing nano(particles) and/or biocides should be assessed in population based studies.
- The properties of chemicals, such as silver-based biocides and of TiO_2 particles, are not essential for the intended use of a face mask, and synthetic fibres suitable for face masks can be produced without TiO_2[22]. Therefore, it needs to be investigated whether the possible health risks associated with biocides and (nano)particles applied in or on face masks do not outweigh their benefits.

Life cycle and end-of-life

- The fate of chemicals released from face masks (and in a broader context from functional textiles) during manipulation and particularly washing, which may lead to environmental issues, requires further research,.
- The end-of-life of face masks (and in a broader context of functional textiles) with (nano)particles, the release and fate of these nanoparticles in the environment and their environmental risks have not been studied in detail yet.
- The potential health risks related to environmental disposal of face masks (and in a broader context of functional textiles) are still unknown. The investigation of the modalities for efficient and safe recycling deserves attention.

Recommendation for policy

The research on the presence of nanoparticles in face masks, as well as the negative opinion of EFSA on the use of TiO_2 nanoparticles in food, underline the importance of developing research on "nanotechnologies and health" in Sciensano, as well as the need to support and reinforce policy on this matter and create opportunities for the private sector in this context. We have observed that:

1. In recent years, a legal framework has been developed at European level regarding the application of nanomaterials in various sectors (food, cosmetics, medicine, consumer goods). Several governmental departments are regularly confronted with nanotechnology-related problems and the specific expertise is lacking to implement and control the new legal and regulatory framework.
2. There is a major lack of insight in the distribution of nanoparticles in the environment, in the exposure of humans and animals and in the impact of these particles on health and biodiversity.
3. There are still many uncertainties regarding the risks of nanotechnology. The analysis of their benefits and the possible adverse effects on health must be carefully balanced.
4. In addition to risks, nanotechnology also offers many opportunities in the context of healthcare, medicines, healthy food, and for a very diverse range of applications.
5. There is currently a high demand from the private sector for the marketing of safe nanotechnologies and from competent authorities to standardize and control these products. At the same time, there are few opportunities for both the authorities and the private sector to obtain high-quality analytical and research results on their technology due to a lack of specialized laboratory capacity.
6. There are few opportunities for the public, the private sector and the authorities in Belgium to obtain correct information about nanotechnologies.
7. Sciensano has a solid national and international reputation and analytical capacity, resulting in various scientific research projects and collaborations.

Therefore, we recommend:

1. To set up an **expertise centre and reference laboratory** in Sciensano for surveillance and health impact evaluation of nanotechnology, to support health policy. This expertise centre should be a state of the art research infrastructure for the evaluation of health risks related to nanotechnology. This infrastructure is required to provide the necessary capacity to the competent authorities for health inspection and control activities, surveillance, risk assessment and scientific advice in a regulatory framework.
2. To establish a **nanotechnology and health information centre** for the public to understand the risks and benefits of nanotechnology.
3. To develop or strengthen the current **standards and regulatory limits** for chemicals including biocides and (nano)particulate products in face-masks based on the safe-by-design principle.

To re-evaluate the safety of biocides in face mask used for the control of infectious diseases and in health care.

In order to address the 1st recommendation, Sciensano would need to develop the following structures urgently:

- The research infrastructure for the analysis and characterization of nanoparticles and nanomaterials should be further expanded;
- The research group on the exposure to chemicals should be reinforced to provide expertise and services with regard to nanoparticle exposure via different routes;
- The research group on risk assessment should be reinforced to provide expertise and services on nanotechnologies and their impact on health.

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